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13. ABSTRACT (Maximum 200 Words)

Women found to have atypical hyperplasia on a breast biopsy are at significantly increased risk for breast cancer. Nipple duct lavage (NDL) is being promoted by some as a new screening test for atypical hyperplasia, but cytological interpretation is a subjective art and experience indicates that the underlying conditions represented by cytological atypia on NDL can range from intraductal papilloma to ductal carcinoma in situ (DCIS). Laboratory studies indicate that methylation of tumor suppressor genes is an early event in breast carcinogenesis and often represents a cell's attempt to defeat cell cycle control. We have shown previously that methylation of RASSF1A or APC in benign breast epithelium correlates with calculated breast cancer risk and the finding of Cyclin D2 methylation is specific for malignant transformation. We are applying these objective methylation tests to cells obtained by NDL from women with breast cancer, women at increased risk of breast cancer, and women at low or average risk of breast cancer. Successful completion of the project will provide new tools for the objective evaluation of breast epithelial cells obtained by NDL in order to accurately risk stratify women and to enhance the early detection of DCIS.

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INTRODUCTION

While the lifetime risk for developing breast cancer is relatively high in all women, a subset of women are at increased risk. Risk can be estimated by computer software using history, pathological findings, previous history of breast cancer and other factors. We have developed custom software for risk estimation of women attending our high risk clinic. However, refinement of risk estimation remains an important goal.

Obtaining tissue for pathological examination usually requires invasive procedures such as needle aspiration or surgical biopsy. A recently developed, minimally invasive technique, nipple duct lavage (NDL) extensively samples the ductal/lobular system (where all breast carcinomas arise) and provides adequate cell numbers for pathologic and laboratory studies.

Several intermediate markers have been utilized for risk assessment. We are testing for aberrant methylation of a panel of genes frequently silenced in breast cancers. In other cancers it has been demonstrated that aberrant methylation commences early during mulitstage carcinogenesis, even in histologically normal epithelium. Thus it is likely that it will be an early event in breast cancer pathogenesis. Utilizing a panel of markers will permit us to utilize data from individual markers as well as a methylation index based on the combined results.

We are performing bilateral NDL on three groups of women (50 in each group) over the course of three years. The groups will consist of a) women with cancer; and women with computer estimated b) high risk and c) low risk. Because data from the contralateral breasts of cancer patients will be analyzed separately, our findings will represent materials from breasts at four levels of risk (beyond risk, very high risk, high risk and low/average risk).

Thus, we will be able to compare and contrast three methods of risk assessment (computer generated modeling/breast cancer diagnosis, cytological examination and aberrant methylation) in women at four levels of risk. We will determine whether cytologic examination of NDL fluids alters computer generated risk assessment. The finding of aberrant methylation in women at low risk without cytologic changes, may help identify a subgroup at increased risk not identified by the other techniques.

Our results will help assess the newly developed technique of NDL, both for cytological diagnosis and utilization of intermediate markers. The data may provide important new information that helps refine breast cancer assessment. Women identified at increased risk will benefit from increased surveillance and chemoprevention. Thus, our proposal is of direct relevance to the clinical management of women at increased risk, and may, ultimately, help reduce the incidence of invasive cancer.

BODY

Recruitment and Sampling

Recruitment of study subjects and performance of nipple duct lavage sampling is behind schedule for meeting our accrual goals in the time specified. For this reason we have requested a no cost extension for a period of one year. To date we have accrued and sampled 115 of the 150 subjects specified by the protocol. Comprehensive risk factor information has been electronically archived for all of the patients as has cytological results for all of the patients. We are batching the methylation assays and do

not foresee any difficulties in completing these rapidly. There have been no serious adverse events.

Finalization of the Panel of Genes to Evaluate

One important objective of the study was to assemble a panel of genes whose methylation status could contribute to risk stratification or early diagnosis of breast cancer. To achieve this, we have been screening archival benign and malignant breast epithelial cells using a panel of six genes. We have determined from this that methylation of RASSF1A or APC correlates with breast cancer risk calculated using the validated Gail model and that methylation of Cyclin D2 occurs in 50% of breast cancers and is a cancer-specific marker. Our final methylation panel in order of priority is: Cyclin D2, APC, RASSF1A, RAR-β, E-Cadherin, H-Cadherin, and TWIST.

Interim Analysis and Findings

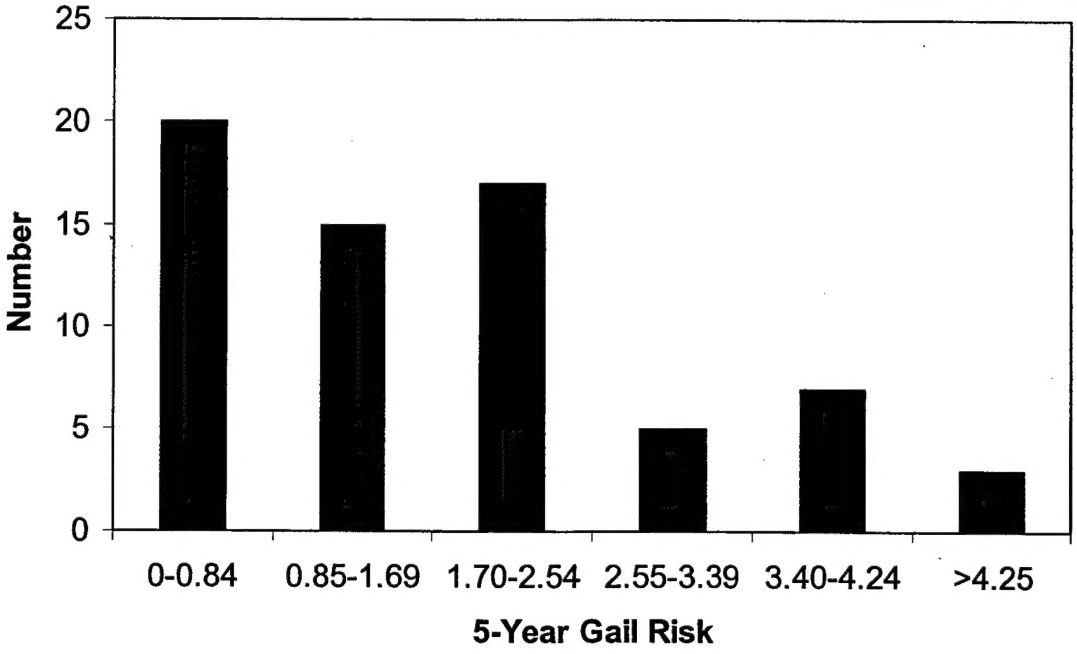
Interim analysis has focused on quality of the samples and cytological findings. We have recently summarized data for the first 108 patients. This summary includes 67 unaffected women and 41 women with breast cancer. The table below summarizes the cytological findings by duct and by breast for these women.

Table 1: Atypical cytology rates by duct and by breast for 108 women.

	Ducts				**Breasts**			
	Acellular	Mild Atypia	Marked Atypia	Any Atypia	Acellular	Mild Atypia	Marked Atypia	Any Atypia
Cancerous Breast	24/60 (40.0)	7/60 (11.7)	8/60 (13.3)	15/60 (25.0)	11/36 (30.6)	5/36 (13.9)	8/36 (22.2)	13/36 (36.1)
Contralateral to Cancer	19/57 (33.3)	6/57 (10.5)	3/57 (5.3)	9/57 (15.8)	10/38 (26.3)	5/38 (13.2)	3/38 (7.9)	8/38 (21.1)
Unaffected Right	35/133 (26.3)	15/133 (11.3)	7/133 (5.3)	22/133 (16.5)	10/67 (14.9)	12/67 (17.9)	5/67 (7.5)	17/67 (25.4)
Unaffected Left	32/127 (25.2)	19/127 (15.0)	6/127 (4.7)	25/127 (21.3)	13/67 (19.4)	12/67 (17.9)	4/67 (6.0)	16/67 (23.9)

Our acellular sample rate and atypia rates are similar to those reported in the literature.

The distribution of calculated breast cancer risk levels for the 67 unaffected women included in Table 1 is shown in the following figure.



Approximately half of the women are considered high risk for breast cancer based on a 5-year Gail risk $\geq$ 1.7%. Atypia rates are essentially the same for low and high risk patients. We have measured the reproducibility of lavage atypia by repeating the lavage in 20 patients. On a per duct basis (55 ducts), the atypia was reproduced in only 13%. On a per patient basis it was reproduced in 50%. Our results suggest that atypia may be physiological rather than pathological in a large number of women. Cytology is too subjective and lacks sufficient specificity for optimal assessment of epithelial cells obtained by nipple duct lavage. It is anticipated that molecular analysis will significantly enhance the characterization of these samples.

KEY RESEARCH ACCOMPLISHMENTS

- Promoter region methylation of RASSF1A and APC in benign breast epithelium correlates with breast cancer risk calculated using a validated mathematical model and may provide an objective measure for risk stratifying duct lavage samples.
- Promoter region methylation of Cyclin D2 is a cancer-specific change that, if applied to duct lavage samples, may contribute to the diagnosis of mammographically occult DCIS.
- Atypia is diagnosed at a similar rate in high and average/intermediate risk women.
- Cytological assessment of duct lavage samples is unable to distinguish between breasts with cancer and breasts without cancer.

REPORTABLE OUTCOMES

Abstract and presentation Society of Surgical Oncology, 2003[1]
Abstract and presentation Association for Academic Surgery, 2003[2]
Abstract and presentation Society of Surgical Oncology, 2004[3]
Abstract and presentation American Society of Breast Surgeons, 2004[4]

Manuscript accepted for publication American Journal of Surgery, 2004[5]

CONCLUSIONS

Preliminary analysis of our data highlights the limitations of cytological assessment as the sole modality for evaluation of nipple duct lavage samples. Preparatory work with methylation markers strongly suggests that the addition of molecular tests to the evaluation of nipple duct lavage samples will provide an objective approach for risk stratification and perhaps for the early detection of mammographically occult DCIS.

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